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THE

MICROSCOPICAL BULLETIN

AND

SCIENCE NEWS

A BI-MONTHLY MAGAZINE

EDITED BY

ALBERT S. BARKER

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29. **Microscopical Petrography,** by Ferd. Zirkel, with plates **6 00**

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"The Effectiveness of Aperture"

will be the title of a series of articles beginning in this number, and continuing during 1897. They are being written expressly for the BULLETIN by Mr. Henry Orford, and will cover the theoretical and practical application of aperture in the microscope objective. A department of "Queries and Answers" in matters pertaining to the microscope will also be maintained during the year.

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NOTES AND COMMENTS.

As the New Year approaches, the BULLETIN renews its good resolutions, and with the desire to be more useful than ever, we invite our readers to send us the questions concerning the construction and manipulation of the microscope to which they cannot readily find answers elsewhere. When we cannot afford space for full treatment of the subject, we can probably indicate where the information may be obtained with minimum expenditure of time and patience.

* * *

We learn that *The Observer* has been having some financial trouble, probably for lack of quick response from the many lovers of natural history for whose pleasure and profit *The Observer* was making extraordinary exertions. We believe that *The Observer* is worth a dollar to every amateur ornithologist, mineralogist, botanist and microscopist in America; and that such a journal may perish for want of a few hundred subscribers while the trashiest "story papers" survive, is a consuming shame to this nation of upwards of sixty millions of inhabitants, many of whom have good common-sense.

* * *

It will probably be admitted that the end justifies the means in the case of Mr. Hankin, who is engaged in bacteriological work in India. It is said that he has been lecturing with gratifying success to priests and leaders, urging them to resist the evils of the god Kali, who appears in the form of bacteria. He exhibits the bacteria under the microscope, explains how they reproduce and thrive (with the aid of the wicked god, of course) and advises sanitation and cleanliness and pure water as the best means of overcoming the effects of these evil visitations. There are places nearer home where a touch of mythology would be useful.

* * *

The Bureau of Vegetable Physiology and Pathology of the Department of Agriculture has just published Dr. Erwin F. Smith's report on "A Bacterial Disease of the Tomato, Eggplant and Irish Potato." This is a technical bulletin "intended mainly to put on record the results of investigations concerning the life history of the organism causing the disease, and to set forth certain suggestions in the way of preventive measures that might be adopted."

This newly-discovered micro-organism, called *Bacillus Solanaceanum*, he believes to be the cause of a large part of the potato rot of the United States.

Numerous infection experiments performed in 1895 and repeated this year have set the parasitic nature of the organism beyond dispute. Some of the peculiarities of the bacillus, as stated by Dr. Smith in a paper read before the Biological Society of Washington, and reported to *Science* by the Secretary, are: Organism motile; forms zoögloea in liquid cultures; does not liquefy gelatin; strictly ærobic, does not produce any gas or any acid when grown in the presence of sugars; produces an abundance of alkali (ammonia) in various media; develops a decided brown pigment when grown in the presence of various sugars (agar cultures, fermentation tubes, potato cultures, etc.); grows readily in the thermostat at 37° C.; thermal death point (ten minutes' exposure) about 52° C. The organism is probably transmitted from diseased to healthy plants by means of insects. In the greenhouses under strict control conditions, very successful infections have been obtained

by means of the Colorado potato beetle (*Doryphora 10-lineata*).

This bulletin (No 12, Division of Vegetable Physiology and Pathology) may be obtained from the Superintendent of Documents, Union Building, Washington, D. C.; the price is 10 cents.

Another important publication of the month is a bulletin on "The Cotton Plant: Its History, Botany, Chemistry, Culture, Enemies and Uses," prepared under the supervision of A. C. True, Ph. D., Director of the Office of Experiment Stations, with an introduction by Charles W. Dabney, Jr. Ph. D., Assistant Secretary of Agriculture. Pp. 433, Pls. IV, Figs. 32.

The chapter on "Insects affecting the Cotton Plant," by L. O. Howard, Ph. D., Entomologist, is also published separately.

* * *

From the Journal of the Royal Microscopical Society we copy the following:—"Eye-piece with Graduated Iris-Diaphragm.* Dr. W. Cowl describes and explains the advantages of an eye-piece provided with an iris-diaphragm. * * * the iris-diaphragm takes the place in the Huyghens' eye-piece of the usual fixed diaphragm. It is provided on its edge with a scale, each division of which corresponds to a doubling of the diameter of the aperture, beginning with 1 mm. The numbers on the scale correspond to the area of the field of view.

Amongst the advantages of the apparatus are—a more acute perception of details in the field of view, owing to the exclusion of neighboring parts of the preparation; the exclusion of unnecessary light; the possibility of varying the magnification by changing the eye-piece without the necessity of adjusting the microscope anew, since the iris-diaphragm only slides in the body-tube up to the diaphragm, i. e. up to the plane of the image formed by the objective."

(Prepared for THE MICROSCOPICAL BULLETIN.)

A PROCESS FOR THE IMPREGNATION OF THE LACUNÆ AND CANALICULI OF BONE WITH FUCHSIN.

In the *Zeitschr. für wiss. Mikroskopie* Herr Max Ruprecht, after reviewing the methods for staining bone recommended by Matschinsky, Ranvier, and Zimmermann, gives a process for the impregnation of bone with fuchsin which he has found to possess many advantages. The article is accompanied by two plates, showing photo-reproductions of four sections prepared by this method.

The dry section of bone, well macerated and completely freed from fat, is filed flat on one side with a sharp file with about 13 cuts per cm. Grinding before staining is avoided, as the pores are thereby obstructed. The section is now cemented with sealing wax by the flat side to a cork or other support, and filed down until the support or sealing wax shows through faintly, when it will be about 0.4 mm. in thickness. It is now detached, and filed on both sides to about 0.3 mm. thickness. Ordinary print can be just read through the dry plate of bone at this point. The scratches of the coarse file are now removed with a finer file (18 to 20 cuts per cm.), and the section is thin enough for staining. Small sections may be as thick as 1 mm. for staining; the larger the section the thinner it must be, but never less than about 0.3 mm. thick.

Small sections of bone need not be cemented to a support for filing, but may be filed down between two files.

When the filing is finished, the pores are opened by scraping with a scalpel. This procedure is credited to Ranvier. It is better than washing, as the section remains dry.

The section is now allowed to lie in ether for a few minutes, then quickly heated on a glass plate or similar support, and returned, hot, to the ether; after remaining here for about five minutes or longer, it is quickly transferred to about 20 cc. of a boiling concentrated solution of diamond fuchsin in absolute alcohol, into which it is introduced flat. When the boiling has been continued for five minutes, the bath is cooled to below 34° C., and subsequently evaporated to dryness at about 70°. The section is dried completely by warming, and the thick layer of fuchsin adherent to it removed by scraping. Care must be taken to avoid contact of the stained section with alcohol or water.

The grinding procedure which now follows, is believed by Herr Ruprecht to be wholly new, and permits sections of any desired size to be ground sufficiently thin, quickly and evenly. Two plates of ground glass, one about the size of the palm of the hand, and the other larger, are required; upon the larger is spread powdered pumice, which is kept moist with a mixture of benzine dehydrated by treatment with anhydrous copper sulphate, with about one-tenth its amount of vaseline oil. The section is placed between the two plates, and ground with an even, heavy pressure. To ascertain if the required thickness has been attained, the section is washed with oil and benzine, and examined under the microscope. When thin enough, all pumice powder is washed off with anhydrous benzine, and the section smoothed on both sides on an Arkansas stone in oil and benzine with the fingers. All oil is now removed by again washing with benzine, and the section dried on blotting paper, and polished on writing paper.

As a mounting medium, colophony dissolved in anhydrous benzol is recommended.

*Anat. Anzeig, XII. (1896) pp. 178-80.

The steps in the procedure may be enumerated as follows:

1. Filing the section to 0.3 mm. thickness, and scraping with a scalpel.
2. Immersion of the dry section in ether. Rapid heating on a glass plate, with immediate return to ether.
3. Transfer of the section, flat, to boiling alcohol solution of diamond fuchsin.
4. Boiling for five minutes, followed by cooling to below 34° C.
5. Evaporation of staining solution to dryness at about 70° C.
6. Removal of coloring matter from the surface of section with knife.
7. Grinding between two ground glass plates with pumice in vaseline oil and anhydrous benzine.
8. Smoothing on an Arkansas stone.
9. Washing with benzine, drying, and polishing with writing paper.
10. Mounting in colophony dissolved in anhydrous benzol.

EFFECTIVENESS OF APERTURE.

BY HENRY ORFORD, PHILADELPHIA.

It was not my intention at first to write a series of articles on the present subject, but one thing and another would crop up and force its way to the front to be explained so that the amount of space at my disposal in a single number of the BULLETIN was totally inadequate to treat, even in the most brief way, the different conditions of an objective for perfect resolution according to its aperture. Power has very little to do with the resolution of striæ on diatoms, for a lens with the whole of its aperture corrected will stand any amount of eye-piecing. Too much is made of a lens that will resolve the striæ of certain diatoms, though under certain circumstances a most indifferent lens can be made to line a certain object where a perfectly corrected and centered objective would fail to resolve it by axial illumination. Several makers are at present engaged in a kind of warfare as to who shall produce the *highest* power $\frac{1}{12}$. A $\frac{1}{12}$ should have an initial magnification of 120, but you will find some that have 160, and in one case a maker has a different power for each aperture. The $\frac{1}{12}$ of 1.25 having an initial magnification of 150 diameters and his $\frac{1}{12}$ 1.30 N.A. 140 initial magnification. This should be discountenanced; it is misleading and makes the various measurement tables of no use whatever. The greatest possible cone of rays in air that can enter an objective has an angle of 180° and such a cone can only enter when the object absolutely touches the plane surface of the lens. Consequently there is no working distance, and every step in the matter of correcting apertures in dry lenses enormously increases the difficulties of the system. In many of the older objectives that had a large aperture most of it could conveniently be shut away, for being uncorrected or imperfectly so, was of very little use. Such a lens with its partially corrected aperture would resolve striæ on diatoms, each in its own way, as we can see by looking over the earlier numbers of transactions of different societies, the image in itself being totally different from that shown by perfectly corrected lenses of the present day. In these times of immersion lenses we gain in illumination, and independence of cover glass to a certain degree, though when we get our large aperture perfectly corrected cover correction will be as necessary as in a dry system, owing to the small likelihood of the front lens, oil and cover being of absolutely the same refraction. This system is more easily corrected owing to no refraction taking place between the front lens and the cover glass, making a large aperture more easily obtainable and as easily corrected as the aperture of a medium dry system. It has more penetration and the increased resolution that comes from large apertures. The simplicity of the correction of the system owing to the lack of refraction between the lens and the cover glass, its comparative freedom of influence from cover glass, has made the immersion lens the most useful made.

These papers will treat on the subject fully (from a maker's point of view) and we shall examine a lens, first to measure its aperture, and then to see its resolving power and image under the different conditions of its aperture, uncorrected, partially corrected and perfectly corrected. Why is it that of two objectives rated as the same aperture, that one will be one-third as bright again as the other? The reason is this, though both lenses would read the same aperture on any measuring instrument, or in any way the aperture of a lens is measured, the one that was only slightly better corrected than the other, would be the brightest, and being better corrected, would give a larger field of critical definition, that is flatter; and the image would seem much thinner.

A lens that is corrected up to 1.1 N.A. and has an extra two degrees partially corrected will give a totally different image from a lens having only an aperture of 1.1 N.A. but perfectly corrected in all its zones. The first will give you an image showing striæ coarse and thick, indifferently illuminated, with, of course, a great amount of under or over correction of spherical aberration apparent, whilst the other will give a a thin, distinct image, the striæ being much finer, though the powers of the objectives are approximately the same. The illumination is better, and altogether the image is more perfect, especially to critical eyes; though to an indifferent observer the one that made the coarse image would go. In the lens corrected in all zones, as far as prac-

tical, you had the image as pure as would be seen by normal vision were the object large enough, but in the other you had an image formed by the part corrected and further resolved by the uncorrected or partially corrected extra aperture. If this aperture were cut away the striæ would gradually get finer and the illumination in some cases would increase, though in the majority of cases the systems would have to be recorrected for distance. It will thus be seen that many objectives of to-day are totally misleading both as to the magnification and the formation of the image. In another way may the markings of diatoms be "resolved." By oblique light, unknown to the manipulator of the instrument. From time to time I have had many objectives to repair, especially Continental ones, owing to their short life, as decomposition generally sets in within a few months of their exit from the factory.*

There is one test and the best, this is resolation by axial illumination without a condenser. If this is easily accomplished, you can be sure you have a good lens; that is considering the image to be good as before stated. Now take a lens and examine a slide of *S. Gemma* for instance, test it with absolutely central light, it probably shows the striæ. Now move the mirror a little oblique, the striæ are well resolved, and the lens under these conditions would be considered a fine lens. Now put the objective under another condition. Suppose we cant the lens, *i. e.*, make the lens itself oblique and put the mirror central, that lens would show the striæ just as well as using oblique light from the mirror. Many hundreds of lenses I have had to repair and now and then I have found an objective true to my lathe, (of the productions of two firms I have never found the least decentering; in fact they were as true as if made on my lathe, as they should be if made conscientiously); but lately I noticed that the majority I had ran false in two different directions. At first I used to put it down to carelessness, but when I took them apart and found the inner cells all ran true, and that it was only between the part that held the system and the adapter that the excentricity was noticeable, and as time after time I had to make a new adapter on which to replace the lenses I had taken out, I put it down that the adapters were probably made by cheap labor or unknowingly on a chuck that was false. I never cared or thought to investigate the matter till some time back, when I had three or four at one time to repair. Years ago I had found even in the faulty construction of the early lenses that one perhaps out of a batch would resolve a certain diatom beautifully, and invariably found that one had a certain amount of excentricity, but though showing those striæ would fail to resolve objects that require central illumination. It is obvious that these lenses are purposely set excentric, and for resolving an object in a dry mount have a great advantage over lenses set in ordinary mounts, and it accounts for their partial performance on objects mounted in balsam.

(To be Continued).

* I am not alluding to apochromatic lenses, though I have examined one lately purchased by a gentleman in this city and made by "the" apochromatic firm that was considerably worse corrected for figure than the average French button, though I must say that it is the first badly corrected lens that I have ever seen of the latter productions from the firm in question; it showed to perfection the "brick red" erroneously stated to be undercorrection, though in reality it is due to bad workmanship, and we look for this even before we focus for an open centre in the star. This would probably escape the examiner if, as in most firms, the star is discarded and an Abbe test plate used in its place. Indeed, I only know of one other maker in this country who uses the mercury globule and understands it.

[For The Microscopical Bulletin.]

DIATOM REMAINS ON THE OCEAN BOTTOM.

BY ARTHUR M. EDWARDS, M. D.

NEWARK, N. J.

As it does not seem to be published except in the original, and as that is very scarce now, not being seen by students of the Bacillaria, the Diatoms, at all, and as it bears strongly on the soundings which I have and which were brought home by the U. S. S. "Tuscarora," and furthermore, as it specially bears on the subject of the genesis of the so-called miocene tertiary deposit of diatomaceæ, which I am endeavoring to clear up, I will be pardoned if I transcribe here the following paper. In this paper on the Diatomaceous vegetation of the Antarctic Ocean, which has become classic now, especially as it was the first that made known the layer of bacillarian shells at the bottom of the ocean, Dr. J. D. Hooker says (Trans. Brit. Ass. Ad. Science, Oxford, 1847, page 83.) "The waters, and especially the newly-formed ice of the whole Antarctic Ocean, between the parallels of 60° and 80° South, were found by the author to abound in an order of organisms, whose true nature had until very recently been disputed; but which the recent discoveries of Mr. Thwaites in England had proved to belong to the vegetable kingdom. This order occurred in such countless myriads as to stain the sea everywhere of a pale ochreous brown, in some cases causing the surface of the ocean, from the locality of the ships, as far as eye could reach, to assume a pale brown color. Though peculiarly abundant in the Icy Sea, these plants are probably uniformly dispersed over the whole ocean; but being invisible from their minuteness, can only be recognized when washed together in masses, and contrasted with some

opaque substance. They were invariably found in the stomachs of *Salpæ* and other sea animals, in all latitudes between that of the north tropic and the highest parallel attained by the Antarctic expedition. Many processes were employed for obtaining the species, which on being brought to Europe were placed in the hands of Prof. Ehrenberg, of Berlin, for examination and publication. A consideration of the results of Prof. Ehrenberg's examination, and of his observations on these plants, has led Dr. Hooker to the following curious results:

1. That a vegetation, very different from *Lichens*, *Mosses*, *etc.*, (orders which had hitherto been supposed to comprise the most Antarctic vegetables) abound in the waters of the Antarctic circle, the species increasing in number with the latitude up to the highest point attained by man.

2. That on this vegetation the whole of the animal kingdom, which swarms in the waters of the Antarctic Ocean, perhaps ultimately depends for its existence. It maintains that balance between the animal and vegetable kingdom which prevails through all other latitudes; probably also purifying the vitiated atmosphere as plants of a higher order do in more temperate regions.

3. The genera and species of *Diatomaceæ* collected within the Antarctic circle are not all peculiar to those latitudes; on the contrary, some of the latter occur in every country between Spitzbergen and Victoria Land. Others, or even some of these, have been recognized by Prof. Ehrenberg as occuring fossil in both Americas, the South of Europe, and the North of Africa, in Tripoli stone and volcanic ashes ejected by living and extinct volcanoes; whilst others again have been found floating in the atmosphere which overhangs the tropical Atlantic; for Mr. Darwin, during the voyage of the "Beagle," collected an impalpable dust which fell on Captain Fitzroy's ship when to the west of the Cape de Verde Islands, and which on examination was proved to consist of the remains of *Diatomaceæ*, including species common in the Antarctic regions.

4. The death and decomposition of this Antarctic vegetation are gradually producing a submarine deposit or bank of vast dimensions. This bank consists mainly of the shields (the siliceous coatings) of *Diatomaceæ* intermixed with *Infusoria* and inorganic matter. Its position is from the 76th to the 78th degree of South latitude, and between the meridians of 165 East and 160 West longitude, thus occupying an area of 400 miles long by 120 wide. All the soundings taken over this deposit were in the finest green mud occasionally mixed with sand, at a depth of between 200 and 400 fathoms. The lead sometimes sank two feet into this pasty deposit, and on examination of what it brought up, showed the bottom to consist in a great measure of the remains of the species living on the surface.

5. This deposit may be regarded as resting on the shores of Victoria Land and Barrier, and hence on the submarine flanks of Mount Erebus, an active volcano upwards of 12,000 feet high. Knowing as we do that *Infusoria*, *Diatomaceæ*, and other organic constituents, enter into the formation of the pumice and ashes of other volcanoes, and are still recognizable in those minerals, it is perhaps not unreasonable to conjecture, that the subterraneous and subaqueous forces, which keep Mount Erebus in activity, may open a direct communication between this Diatomaceous deposit and its volcanic fires.

6. The *Diatomaceous* deposit flanks the whole length of Victoria Barrier, a glacier of ice 400 miles long, whose seaward edge floats in the ocean, whilst its landward is extended in one continuous sweep from the crater of Mount Erebus, and other mountains of Victoria Land, to the sea. The progressive motion of such a glacier, and accumulations of snow on its surface must result in its interference with the deposit in question, which, if ever raised above the surface of the ocean, would present a striped bed of rock which had been subjected to the most violent disturbance."

ENDOCYSTS OF DIATOMS.

OCCURRENCE IN A NEW SPECIES RECORDED BY MR. THOMAS COMBER.

In the October number of the Journal of the Royal Microscopical Society, Mr. Thos. Comber has a paper (with photographic illustrations) "On the Occurrence of Endocysts in the Genus Thalassiosira." Attention is called to the observation by Mr. Lauder, in 1864, of the formation, within the frustule of various species of *Bacteriastrum* and *Chætoceros*, of what he termed "a gonidium or sporangium, consisting of a cell with two rounded ends, and a connecting hoop, one end being smaller than the other." He supposed this to be a phase of a special method of reproduction, and described it as originating in the condensed endochrome of the frustule secreting a siliceous envelope, more or less spinous, within which highly refractive globules were formed. The frustules then break up, and set free the enclosed bodies. "The contents of the sporangium soon escape;" but he was not able to follow out the further processes they undergo towards the reproduction of the original form. He suggested that many, if not all, the species in the genera *Goniothecium*, *Ompthalotheca* and *Hercotheca*, and perhaps in *Dicladia*, *Periptera* and *Syndendrium*, would "turn out to be sporangial bodies of species of Chætoceros."

"To some extent," says Mr. Comber, "this surmise has proved correct. In the 'Challenger' collections, for instance, Count

Castracane found within the frustules of a *Chætoceros*, the form *Dicladia capreolus* Ehr., well known from its frequent occurrence in guano. Other instances of the formation of these bodies have been recorded by Dr. Cleve, who, however, proposes for them the term 'endocysts;' and this appears to be a better name as their real function is still quite uncertain."

The object of Mr. Comber's paper is to place on record the occurrence of endocysts in the genus *Thalassiosira*, not in the Arctic species *T. Nordenskioldii* for which the genus was constituted by Dr. Cleve in 1873, but in a hitherto undescribed species recently received by Mr. Comber from a point in the Antarctic Ocean. For this species Mr. Comber proposes the name *T. Antarctica*. Although the endocysts are frequent in the material, no instance has been observed of their undergoing subdivision.

DESCRIPTION OF SPECIES.

Thalassiosira antarctica.—Filament composed of 3-20 frustules. Diameter of valve 0.016-056 mm., surface slightly convex. Puncta rounded, of equal size, 20 in 0 01 mm., arranged in radiating, frequently bifurcate rows, about 18 in 0.01 mm. Centre frequently with one or two large granules, or short spines. Marginal apiculi small, inconspicuous, numerous, about 8 in 0.01 mm., sometimes arranged in two rows. Endocyst lenticular. Areoles irregularly hexagonal, 7 in 0.01 mm., decreasing in size near centre and towards margin, arranged in radiating rows. Inter-areolar apiculi numerous, either singly or in groups of two or three. Marginal spines conspicuous, about 3 in 0.01 mm.

South Shetland Islands, Antarctic Ocean.

The ordinary valves resemble those of the Arctic species *Coscinodiscus hyalinus* Grun. and *C. bioculatus* Grun., although these are described as having fasciculate markings.

(Translated for THE MICROSCOPICAL BULLETIN.)

NEW OBSERVATIONS ON POTATO-SCAB.

BY E. ROZE.

In a preceding note* I had the honor to make known to the Academy what was the first cause of this disease. This year I have been able to follow the various developments of the disease, whose propagation is singularly rapid, for the garden in which I had arranged to make experiments on this subject, in furnishing me with large pots in which to make cultures under control with scabby tubers, gave me a striking example. A hundred potato plants of different varieties, which were growing in this garden, were all, from this cause, more or less contaminated, though the pots which contained the scabby tubers were at a distance of more than twelve metres from some of these plants, and were arranged in rows separated by walks a metre in width. Although I did not make any precise experiments to verify the special role which is played by the sufficiently numerous worms in this general contamination, I feel justified in regarding them as having been the agents of transportation of the *Micrococcus pellicidus* to the scabby tubers, the viscidity of their bristly segments being apt it seems to me, to cause them to become coated with the mucus of the micrococcus; and more than this, I have not remarked in this garden any other excavating animal capable of producing this dissemination; and where the tubers were uprooted, numbers of worms were always found among them.

However, I now resume my observations on what I have been thus enabled to prove without difficulty.

The potato scab has three stages of development; the first stage begins invariably by the small punctiform pustules obtained by me in my first experiments in the Spring; on the red varieties these minute pustules are whitish; they are brownish on the yellow and violet varieties, although on varieties streaked with red, they resemble dots of carmine, as the result of a special formation of coloring matter with which the tuber seems to offer resistance at the points of attack.

The second stage is characterized by the presence of cracks, generally of little depth, which radiate more or less regularly around the primordial punctiform pustules. These cracks are brownish on all varieties.

Finally, in the third stage, the brown cracks become deeper, lengthen, and even in some cases rejoin each other to such an extent as to cover the whole surface of the tubers. The early varieties ordinarily exhibit the disease only in its first stage; the medium-early exhibit it already in the second stage, while the late varieties exhibit it in either the second or third stage. The development taken successively by the micrococcus has seemed to me to coincide, indeed, with the rains of Spring, of Summer, and of Autumn, and there appears to be a direct relation between increased humidity of the soil, and the progress of the disease.

Finally, this disease of potato scab, wholly superficial, is hardly visible in its first stage, is a little more distinct in the second stage, but it rarely, before the third stage, begins to be really injurious.

I have made microscopical researches as to the possible causes which could coöperate with the micrococcus in producing the characteristic brownish cracks of the disease, and contrary to my expectation I have been led to recognize that the *Bacterium Bolleyi* does not produce all of the effects which I

*Translation appeared in the June number of THE MICROSCOPICAL BULLETIN.

thought should be attributed to it. I have found it, but rarely in the deep cracks, and always in the zoöglœa state. Its action seems very limited, for I have never seen it completely occupy the cells nor perceptibly attack the tissues.

Nor does the *Micrococcus pellicidus* present itself in large masses; it contents itself with passing through the cell walls, and living at the expense of the protoplasmic contents of the cells. It thus introduces mortification into all of the epidermal tissue which it penetrates, and is seldom to be seen except on the walls of cells recently invaded.

Another competent cause has been announced as being produced by a mucedine which I long endeavored in vain to discover; it is the *Oospora scabies* of Dr. Thaxter. At length, however, I was able to demonstrate its presence in my microscopical preparations, made with extremely small portions of degenerated and spongy tissue detached from the cracks. But this tardy discovery put me on the track of entirely new researches, for I was at first much surprised not to find this Oospora, except when the scabby tubers exhibited at the same time another disease, the Rhizoctone of the Potato.*—*Comptes Rendus.*

(Prepared for THE MICROSCOPICAL BULLETIN.)

TECHNIC OF PARAFFIN SECTIONS.

Dr. H. Albrecht and Dr. O. Stoerk, in an article in the *Zeitschrift fur wiss. Mikroskopie*, after reviewing the various methods commonly used for the attachment of paraffin sections to the slide, give a method which they have worked out, by which it is possible, they claim, to completely spread out sections as large as may be desired, to affix them securely and expeditiously, and to avoid shrinkage as entirely as with the clove-oil-collodion method.

The procedure is as follows: The slide is first breathed upon, to secure the adhesion of water, a drop of which is placed upon it and spread out so as to form a rather thin layer. Upon this the sections are laid, and, by breathing upon them repeatedly, they are completely spread out. The superfluous water is allowed to run off as soon as the sections are arranged.

The sections are now firmly pressed against the slide with a pad of filter-paper, the pressure being applied with the thumb. The filter-paper used is perfectly smooth, and leaves no fibres behind; the side of the pad applied to the sections is moistened with a few drops of absolute alcohol. In the subsequent manipulation care must be taken that the sections do not become in the least dried, as otherwise they will shrink.

The next step is the solution of the paraffin in xylol, which is followed by displacement of the xylol with absolute alcohol.

Two or three drops of a very dilute solution of celloidin are now caused to flow over the sections, and the slide is held inclined to run off the excess.

Finally, the slide is flooded for a couple of seconds with 95 per cent. alcohol, and transferred to water; the sections are now in condition for such further treatment as may be desired, at the conclusion of which the superfluous celloidin on the slide is removed by simply wiping with a cloth, without disturbing that part of the film immediately adjacent to the preparation.

After the treatment with celloidin, absolute alcohol and other reagents in which celloidin is soluble must, of course, be avoided.

The celloidin solution used must be quite dilute; it is prepared as follows:

Dilute celloidin solution is added, drop by drop, to pure ether, until a cloudy turbidity results. Absolute alcohol is now added, in quantity slightly greater than is required to effect the disappearance of the turbidity.

Drs. Albrecht and Stoerk have found their process absolutely trustworthy, except with material treated with osmic acid. When dealing with such material, they first prepare the slide with a mixture of egg albumen and glycerin, then apply the drop of water, and proceed as given above.

(Translated for THE MICROSCOPICAL BULLETIN.)

NOTE ON THE DEVELOPMENT OF BLACK ROT OF THE GRAPE. (GUIGNARDIA BIDWELLII.)

BY P. VIALA.

The forms of reproduction of the *Guignardia Bidwellii*, the cause of the Black Rot of the Grape, are, like those of most of the Pyrenomycetes, very complex and varied. In various publications we have noticed the pycnidia, the spermagonia, the perithecia, the conidiophores, simple or pycnidial sclerotia, and mycelial spores analogous to the chlamydospores. The pycnidia play the most important role as the organs of propagation of this important disease of the grape during the period of vegetation, or as organs of perpetuation of the parasite, with the sclerotia and perithecia, through the Winter. The annual reinvasions are the result of the development, in the Spring, of the perithecia and their sporidia from the simple or pycnidial sclerotia, but result also from the pycnidia which are preserved uninjured, with their stylospores,

*The observations which I have made in this connection will be the subject of another explanatory note treating of the relations between the scab disease and the Rhizoctone.

often in two or three hours and at most in six or eight hours.
from Autumn until Spring; at this time numerous pycnidia may be found full of well developed stylospores. The chlamydospores form only under the abnormal conditions of artificial culture, and are never observed, as far as we have been able to determine this year, in the normal vegetative state of the fungus and the grape.

The conidiophores had been noticed only exceptionally,* and as arising from the sclerotia of dry grapes, after the period of vegetative rest, and only in artificial cultures; they had never been observed in a natural state in the vineyards. The great and disastrous invasion of Black Rot in 1896, in the Department of Gers, has enabled me to observe the conidiophores in large numbers and very frequently, and to learn the important role which they play as organs of rapid and far-reaching propagation of the parasite; as the cause of the intensity and gravity of the disease under the conditions of heat and humidity most favorable to the fungus. In general, however, the pycnidia, with their stylospores, are the most common elements for the multiplication of the *Guignardia Bidwellii.*

The conidiophores develop, when the Black Rot is fully established, from the internal mycelium in the grapes or from the pycnidia which have already discharged their stylospores. They may thus be obtained in artificial cultures, and they are seen to be very numerous in the vineyards, on grapes which bear numerous pustules and which have reached their normal size before *la véraison.* The vegetative activity of *G. Bidwellii* is so great in the month of August, with heavy weather (hot and moist), that the mycelium forms, particularly near the skin, a thick tangled mass of filaments, which—as we have demonstrated for the first time—produce two to three superposed stages of pycnidial conceptacles, which successively produce at the surface, the stalks of their stylospores. In the same intense vegetative condition of the fungus one sees certain subepidermic pellets of mycelium, condensed like those from which the pycnidia originate, open out upon the surface of the infected grape in numerous whitish tufts, which consist of conidiophores. In other cases, and often on the same grapes, the pycnidia, emptied of their stylospores, are compressed toward the exterior; their mouths burst open and enlarge sufficiently to expose the interior of the conceptacle; the basidia then lengthen into conidiophores which form whitish islets analogous to the preceding. The development of the conidiophores† may be followed in artificial cultures; they form

The experimental proof of the relation between these conidiophores and the *G. Bidwellii* had not yet been given. In inoculating the young conidia, or those produced in artificial culture, on healthy grapes, we have produced all of the characteristic changes of the Black Rot.

If these inoculated grapes are kept in a moist atmosphere at a temperature of from 30° to 35°C., they are invaded by the mycelium and completely browned in eight or ten hours; twelve or fifteen hours later the tufts of mycelium become condensed on the surface of the skin, and the pycnidia develop. If these grapes, attached to the vine, can be kept under these conditions, the pycnidia open, emit their stalks of stylospores, then expand and their basidia develop into conidiophores.‡ The inoculations by the conidia or the stylospores, succeed best, if we first of all, and for five to ten minutes, plunge the grapes into almost boiling water, or better, into acidulated hot water. I will call attention in an another article to some phenomena of the White Rot of the Grape (*Charrinia diplodiella*), and certain facts deduced from these latter experiments. The inoculations by the conidiophores, as I have been able to assure myself, determine a more rapid alteration, or at least a more rapid beginning of alteration, than the inoculation by the stylospores; the conidia germinate more readily and sooner than the stylospores. A grape inoculated by the conidia is wholly brown at the end of eight to ten hours; inoculated with the stylospores, the first signs of alteration do not appear until six or eight hours after, and the grape under the most favorable conditions, is not entirely altered until two or three days later.

These experiments and the observations of the tufts of conidiophores in the vineyards of Gers explain certain exceptionally sudden and rapid invasions of the vines of the South-west.—*Comptes Rendus*, Nov. 23, 1896.

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*Viala et Ravaz, Nouvelles espèces de *Phoma* (Bull. de la Société botanique de France, 1886).

†The conidiophores measure from 150 to 180 μ. in height. The filament is cylindrical, partitioned and swollen at the base. It divides into two, three or four branches, which are also swollen at their point of insertion, and separated by a partition. These secondary branches growing more slender, ramify into two, three or four stipes of uniform length, each of which produces a conidium at its pointed summit. The conidia are ovoid (5 μ by 2 to 3 μ) granular, colorless and transparent, with an extremely delicate enclosing membrane. In our experiments the conidia germinated even in solutions of cupric sulphate of strengths which stopped the progress of the spores of mildew; but their germination was prevented by cupric solutions in which the stylospores, nevertheless, continued to vegetate.

‡In submitting certain of these grapes by special process (circulation of air with progressive action of calcium chloride) to gradual dessication, the affected grapes become wrinkled, the pycnidia not yet open remain closed with their stylospores in the interior, and we obtain at the end of fifteen or twenty days, the production of sclerotia from the developing tufts of mycelium.

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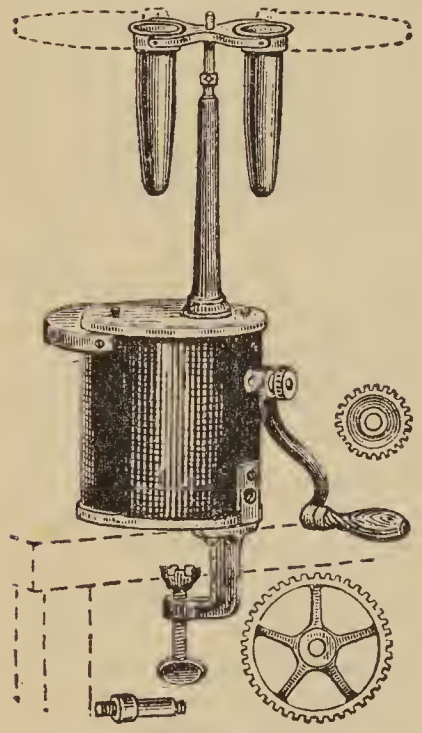

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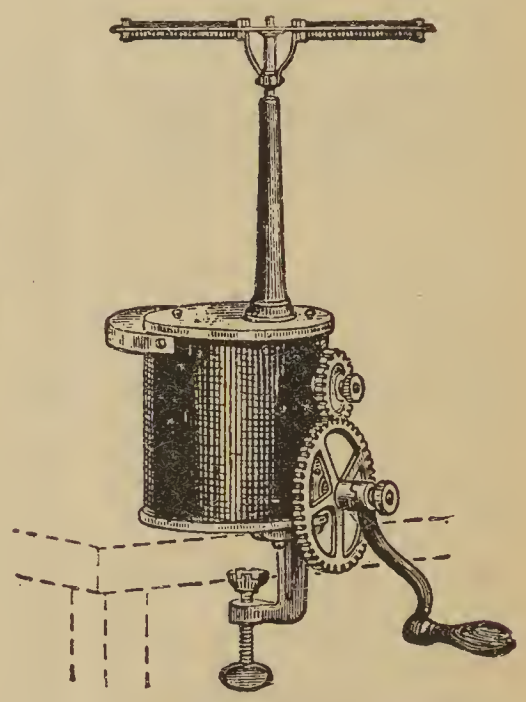

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